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ABSTRACT

Commercial nurseries in BC that grow reforestation stock receive advertising from companies selling ectomycorrhizal inoculum. Since nurseries are always interested in improving the value of their stock while minimizing costs, there are often questions about the value of inoculation with ectomycorrhizal fungi. While it is indisputable that conifers form mycorrhizae in BC forests and benefit from these associations when nutrients and water are limiting, the evidence for benefits of inoculation with ectomycorrhizal fungi in the nursery is not so clear. In 1996, we initiated a study of the inoculation of conifer seedlings with ectomycorrhizal inoculants with funding from Forest Renewal BC. There were two main parts of the study: to determine mycorrhizal status of standard commercial stock and the effects of inoculation in the nursery; and to determine the effects of nursery inoculation on subsequent field performance on cutblocks, roads and landings. All commercial forest nurseries in BC were invited to participate in the study and many did. We have established field trials with inoculated seedlings near Enderby, Prince George, and Fort St. James. The trials compare the growth of inoculated and control seedlings on standard reforestation sites (cutblocks), unrehabilitated landings or roads , rehabilitated landings or roads, and burn piles. In general, our results demonstrated that commercial lodgepole pine stock is highly mycorrhizal, interior spruce moderately so, and interior Douglas-fir very poorly mycorrhizal without inoculation. Inoculations with commercially available inoculants gave variables response in mycorrhizal colonization from year to year, which means that commercial nurseries would not uniformly succeed in improving mycorrhizal development through inoculation. Field responses to inoculation and site rehabilitation are not yet determined since at least another growing season of data should be collected before conclusions can be drawn.

KEYWORDS

Lodgepole pine, interior spruce, interior Douglas-fir, container stock, ectomycorrhiza, PGPR

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INTRODUCTION

Rationale for conducting the project

In nature, all forest trees form intimate associations between their roots and a wide variety of micro-organisms, some of which are beneficial, perhaps even essential, to the plants. On severely disturbed reforestation sites, such as landings, the community of these naturally-occurring root associated (rhizosphere) micro-organisms is dramatically reduced. Various approaches to the reclamation of landings are possible from mechanical alteration of soil physical conditions through deep plowing to amendment of the landings with organic matter. Direct enhancement of beneficial rhizosphere organisms is a new approach that holds considerable promise in promoting survival and early growth of planted conifer seedlings. Two general approaches to the application of this biotechnology are possible: inoculation in the nursery and inoculation at time of planting. This research proposal focuses on the use of commercially-available biological inoculants in improving survival and growth of conifer seedlings on reclaimed landings in comparison with standard reforestation sites.

Beneficial rhizosphere micro-organisms that are currently commercially available include fungi and bacteria. The most familiar and tested beneficial rhizosphere fungi are the mycorrhizal fungi. Rhizosphere bacteria and microfungi are known to improve plant growth through a variety of mechanisms from associative nitrogen fixation to P solubilization and production of chemicals active in iron chelation. Field tests of some of these micro-organisms have produced promising results on conifer seedlings. Mycorrhizae provide many benefits to seedlings. The most important is that their mycelium extends and connects the root systems of their host plants with micropores in the soil and forest floor. This network greatly increases the ability of ectomycorrhizal plants to absorb nutrients and moisture from the soil. Mycorrhizal fungi also produce growth hormones inducing branching of feeder roots, resulting in highly branched root systems (Slankis 1973, Rupp and Mudge 1985), thus enlarging their total absorptive surface. When mycorrhizal seedlings are planted, the mycorrhizal fungi of the seedlings rapidly establish contact with the soil, aid seedlings in overcoming moisture and nutrient stress, and decrease transplant shock (Perry et al. 1987), which is important for survival and growth of outplanted seedlings (Bjorkman 1962; Villeneuve et al. 1991), especially on severely disturbed sites. The beneficial effect of inoculating out planted seedlings with mycorrhizal fungi (Mikola 1973; Marx et al. 1992) is well documented but is not without problems.

Mycorrhizae also occur on nursery-grown seedlings. Depending on nursery location and management, seedlings can become mycorrhizal without artificial inoculation (Molina and Trappe 1984; Hunt 1992) but this varies tremendously due to differences in requirements of different host and fungus combinations. Physical, chemical and biological factors affect mycorrhiza formation in nurseries such as substrate pH, soil microbiological activity, toxicity of fungicides, and substrate fertility (Linderman 1987; Hunt 1992; Mikola 1973). Each fungus is adapted to a given set of ecological conditions and contains numerous ecotypes. Failures in past attempts to improve survival and growth of nursery seedlings after outplanting by inoculating seedlings with mycorrhizal fungi (Shaw et al. 1987; Castellano and Trappe 1991) stem from these factors. This generalization holds for biological inoculants of all types, whether bacterial or fungal.

Status of the research knowledge at the project start

Little research has focused on mycorrhizae of nursery seedlings in BC and even less on other rhizosphere microorganisms. Of the thirty commercial forest nurseries only one, Heffley Reforestation Centre Ltd. (since closed), has done effective research on mycorrhizae (Hunt 1991,

1992). Hunt (1991) found that five species of mycorrhizal fungi occurred on the seedlings in this nursery and that mycorrhizal plants had high quality root systems. He also demonstrated in field trials that the mycorrhizal plants had better early growth than nonmycorrhizal plants (Hunt 1992).

In BC nurseries, managers are looking for ways to improve seedling quality for reforestation and reclamation. Without knowing what the mycorrhizal status of their seedlings is, some nurseries have already invested in commercial inoculum from the United States (personal communication with nurseries) to produce mycorrhizal seedlings. This commercial product might not be suitable since mycorrhizal fungi require specific and different host plants, nursery management, and ecological conditions (Mikola 1973; Perry et al. 1987). There is a need for quality assessment of forest nursery seedlings in terms of types and abundance of mycorrhizae to form a baseline for the development of procedures and for the efficient testing of commercial biological inoculants. Nursery managers are also confronted with advertising that pushes biological inoculatants but they are not provided with research results evaluating the value of these inoculants or reliable procedures for the successful use of these products. Further, they have no reliable information on whether this type of inoculation has any value in the long term in survival or growth of the nursery stock on standard reforestation sites or reclamation sites where success of planting is even more critical.

Project Objectives

1. Quantify and describe the different types of mycorrhizae common to forest tree seedlings grown in nurseries in British Columbia.

2. Inoculate nursery-grown conifer seedlings with a range of commercially available biological inoculants and inoculants under development to assess growth effects in the nursery and after out-planting.

3. Develop cultural procedures for commercial nurseries that enhance the effects of biological inoculants.

4. Compare the relative survival and growth of nursery seedlings inoculated in the nursery or at time of planting on standard reforestation sites.

5. Compare the relative survival and growth of nursery seedlings inoculated in the nursery or at time of planting on reclamation sites.

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METHODS

Commercial nursery stock mycorrhizal status

For 3 years, commercial nurseries in BC donated uninoculated Douglas-fir, lodgepole pine, and interior spruce stock for the assessment of mycorrhizal status. All of the stock had been grown in styroblocks using standard nursery practices. For each species, each nursery sent us 10 - 20 seedlings.

Mycorrhizal status was assessed after roots were cleared and stained since this is the only practical means by which minimally developed mycorrhizae can be detected (Roth and Berch 1992). Determination of percent mycorrhizal colonization was based on the gridline intercept method (Giovanetti and Mosse 1980).

Inoculation with commercially available ectomycorrhizal inoculants

Seeds of interior Douglas-fir, interior lodgepole pine, and interior spruce were stratified in January and February at the Tree Seed Centre, Tree Improvement Branch, and sown at Extension Services, Surrey, in April. After germination, fertilizer was applied following standard nursery procedure.

At approximately 8 weeks of age, Douglas-fir inoculated with mycelial slurry of *Laccaria laccata* and interior spruce and lodgepole pine with *Hebeloma longicaudatum* supplied by Mikro-tek following procedures described and demonstrated by Mikro-tek in Timmins, ON. The mycelial slurry was fragmented using a blender, diluted in tap water, and applied to seedlings in styroblocks using a watering can. At the same time, Douglas-fir was inoculated with *Rhizopogon parksii*, interior spruce with *Rhizopogon subcaerulescens*, and lodgepole pine with *Rhizopogon rubescens*. The *Rhizopogon* inoculum was supplied by Mycorrhizal Applications, Grants Pass OR. In 1999, we also inoculated Douglas-fir and lodgepole pine with *Rhizopogon parksii* collected near Williams Lake BC by Bill Chapman, Cariboo Forest Region.

Plants were lifted in November. At lifting, 40 seedlings from each species and treatment were selected systematically with equal number of samples taken from each styroblock; no samples were taken from the edge rows. They were placed in cold storage until examined and mycorrhizal status assessed using the method described above.

Outplanting tests

Seedlings that have either been inoculated in the nursery or inoculated at time of planting will be outplanted onto a range of sites from standard reforestation sites to reclaimed landings. A minimum of three standard reforestation sites and adjacent reclaimed landings will be randomly planted with treated seedlings of the conifer species. Standard noninoculated nursery stock will serve as controls. In the first and second growing seasons, we will evaluate seedling survival and/or measure seedling growth and nutrient concentration.

New study to improve rooting and mycorrhiza development of Fdi

Data we have collected over the last few years from commercial nurseries in the province have indicated that Fdi, in contrast to Pl and Sx, forms very little mycorrhizae either with indigenous or inoculated mycorrhizal fungi. Past inoculations at Extension Services have demonstrated that mycorrhiza formation by Fdi is possible, although the extent of colonization seems never to be as high as in Pl or Sx. We have also observed that Fdi forms very few fine feeder roots in the nursery. Because mycorrhizae cannot form if fine roots don't develop, we believe there is a relationship between the two observations.

Many researchers, including Hunt (1991) in British Columbia, have demonstrated that there is a relationship between growing conditions, especially fertilizer regime and aeration of the substrate, and mycorrhiza development in the nursery. There is concern about the poor mycorrhization of Fdi stock and the poor initial growth of this species on hot dry sites in the Interior. Therefore, we initiated a study at Extension Services to determine whether fine root development and mycorrhization of Fdi can be improved by altering substrate aeration and fertilizer regime. The design includes three different growing substrates (fine, medium and coarse textured) and 2 different fertilizer regimes (soluble fertilizer only, soluble and slow-release fertilizer combined).

These seedlings have been grown by Extension Services and will be lifted by them in November 2001. At that time, we will assess overall seedling growth as well as mycorrhizal colonization and root system development.

New study to assess native mycorrhiza potential of Miriam Creek site

Francois Teste is collecting data on seedling growth and mycorrhiza development of Fdi stock raised by Riverside. For this study, seedlings were planted in soil collected from the cutblocks and landings at Miriam Creek in May 2001. The soils were mixed with Turface and peat and the seedlings grown in the mixture in the glasshouse for approximately 3 months. Diversity and abundance of native ectomycorrhizal fungi is been determined for 5 seedlings and percent colonization for 10 seedlings per treatment combination. The results of this study of mycorrhization potential will be compared with results for the field-grown seedlings. In addition, this study is providing invaluable training for Francois prior to assessment of the field-grown plants.

DISCUSSION

Progress of the research relative to the approved workplan

Quarter	Task/Activity	Status and reasons for change
1 Sept - Nov	Measure Fdi growth at Miriam Creek	Done
	Collect Fdi from Miriam Creek for assessment of mycorrhizae, growth, and nutrient content	Done
	Continue native mycorrhiza potential assessment of Miriam Creek site	In progress
	Sample preparation and lab analysis of soil samples for bulk density, water retention, and soil chemistry	In progress
2 Dec - Feb	Complete native mycorrhiza potential assessment of Miriam Creek site	Done
	Begin assessment of Fdi raised at Extension Services for test of planting mix and fertilizer regime.	Done
	Prepare article for Seed and Seedling Extension News	Done
	Prepare referred journal article on mycorrhizal status of commercial nurseries	In progress
3 Mar - May		Because of the early termination of Forest Renewal BC, so of these tasks will have to be completed after the termination date.
	Complete assessment of Fdi raised at Extension Services for test of planting mix and fertilizer regime.	Done
	Complete assessment of Fdi from Miriam Creek for assessment of mycorrhizae, growth, and nutrient content	In progress

	Measure Pli at Bear Lake	To be done
	Measure Pli, Sx, and Fdi at Fort St. James	To be done
	Data analysis and interpretations for soil lab results, soil climate monitoring	To be done
4 Jun - Aug	Commence writing thesis on Fdi from Miriam Creek including native mycorrhiza potential, field mycorrhizae, growth, and nutrient content.	To be done
	Complete data analysis and interpretation for Bear Lake, Miriam Creek and Fort St. James trials	To be done
	Prepare referred journal article on Bear Lake and Miriam creek Trials	To be done

Advance on research knowledge

Standard Nursery Stock

In all 3 years, lodgepole pine (Pl) seedlings showed the highest spontaneous mycorrhizal colonization rates in commercial nurseries (Table 1), followed by hybrid spruce (Sx), and finally interior Douglas-fir (Fd). In all 3 years, Fdi was virtually nonmycorrhizal at time of lifting regardless of which nursery raised it.

Table 1. Mycorrhizal colonization of uninoculated conifer seedlings raised in container nurseries in 1997 – 1999 in British Columbia.

	Sx		Pl		Fdi	
Year	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
1997	45	4 - 83	59	12 - 93	9	0 - 30
1998	64	22 - 89	68	13 - 96	2	0 - 8
1999	42	15 - 71	75	41 - 85	1	0 - 8

The lack of colonization in Douglas-fir is of real interest since there are many anecdotal reports of poor early performance of Douglas-fir seedlings on hot, dry interior sites. Any improvements in root and mycorrhiza development for Douglas-fir in the nursery that translated into improved early growth in the field would be of real value because improved early growth could directly affect time to free-growing and green-up.

For all 3 conifer species, the range of mean colonization for individual nurseries varied tremendously. For instance, in 1997 the mean colonization for Sx ranged from 4 – 83% which means that Sx from one of the nurseries had only 4% of the fine roots tips colonized by mycorrhizal fungi and another had 83%. This variation can be explained by many factors from genetic differences (seedlot) to cultural differences.

Inoculated Nursery Stock

Response of mycorrhizal status to inoculation in the nursery at Extension Services varied tremendously from year to year (Table 2). In 1998, inoculation of lodgepole pine with *Hebeloma longicaudum* from Microtek did not improve colonization. In 1999, inoculation with *H. longicaudum* or *Rhizopogon parksii* from Oregon or BC improved colonization. In 2000, seedlings inoculated with *Rhizopogon rubescens* had the same level of colonization as the uninoculated controls but seedlings inoculated with *H. longicaudum* had reduced colonization compared to the others.

For some reason, in 2000, virtually all inoculated seedlings of all 3 conifer species had lower colonization than the uninoculated control seedlings. The results from 1998 and 1999 had encouraged us to think that colonization of Douglas-fir could be improved with inoculation. However, in the 2000 inoculation neither ectomycorrhizal fungus improved colonization. This variability of response to inoculation from year to year is a problem for anyone thinking about buying inoculum.

Table 2. Mycorrhizal status of conifer seedlings raised in container nursery at Extension Services and inoculated with two commercial and one experimental ectomycorrhizal fungus inoculant

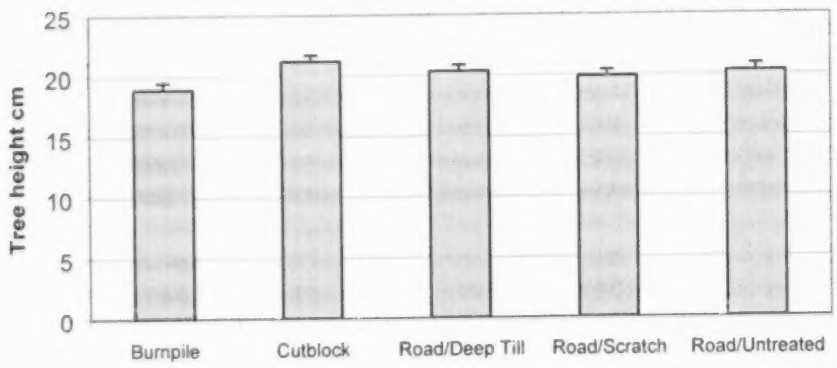
Commercial Source	Fungal species	1998			1999		2000		
		Pl	Sx	Fdi	Pl	Fdi	Pl	Sx	Fdi
	Uninoculated control	92 a	61 a	0 b	76 b	1 b	88 a	61 a	5 a
Mycorrhizal Applications	Rhizopogon parksii	-	-	-	97 a	38 a	-	-	1 b
	Rhizopogon rubescens	-	-	-	-	-	90 a	-	-
	Rhizopogon subcaerulescens	-	-	-	-	-	-	47 b	-
Mikrotek	Hebeloma longicaudum	93 a	75 a	-	98 a	-	75 b	51 b	-
	Laccaria laccata	-	-	7 a	-	38 a	-	-	0 b
Experimental	Rhizopogon parksii	-	-	-	97 a	33 a	-	-	-

Assessment of existing trials

Lodgepole pine seedling growth at Bear Lake

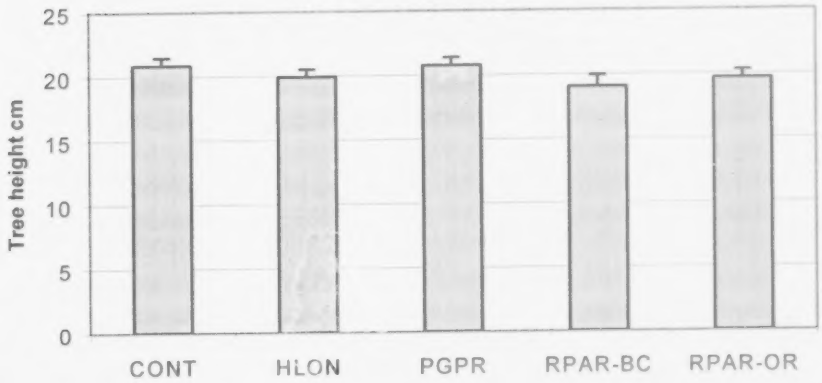
Seedling survival and growth were assessed in mid-September, 2000. Survival was virtually 100%. After only one year in the field, shoot height differences among soil rehabilitation and inoculation treatments were small. Lodgepole pine seedling height was significantly greater on the cutblock than on the other treatments and significantly lower on the burn pile (Figure 1).

Figure 1. Bear Lake: lodgepole pine seedling height after 1 year



Height of control and PGPR lodgepole pine seedlings was greater than for the mycorrhiza-inoculated plants (Figure 2) including *Hebeloma longicaudatum* (HLON), and

Figure 2. Bear Lake: lodgepole pine seedling height after 1 year



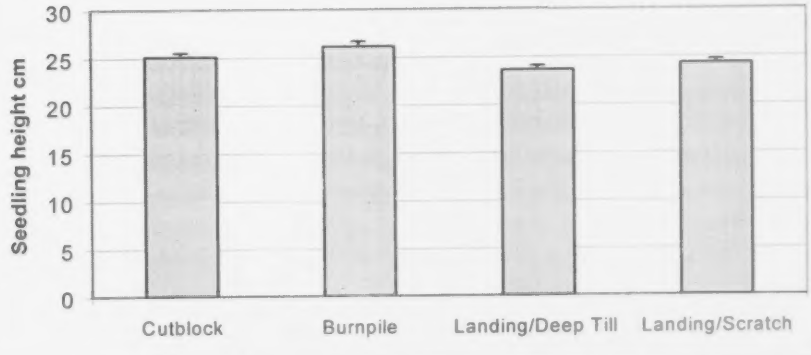
Rhizopogon parksii from the commercial source in Oregon (RPAR-OR) and from a native collection from BC (RPAR-BC).

Year 2 assessment of survival and growth at Bear Lake will be carried out in May 2002 before growth recommences. An advantage of waiting until early spring is that survival assessment takes into account another winter.

Douglas-fir seedling growth at Miriam Creek

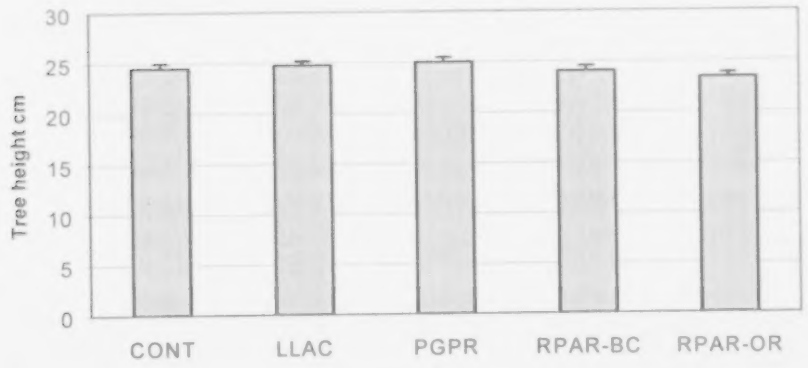
After only one year in the field, shoot height differences among soil rehabilitation and inoculation treatments were small. However, in contrast to the situation with lodgepole pine at Bear Lake, Douglas-fir seedlings planted in the burn pile were significantly taller than seedlings on the other treatments (Figure 3).

Figure 3. Miriam Creek: Douglas-fir seedling height after 1 year



Seedling height did not differ between uninoculated controls and the rest of the treatments, however the plant growth promoting rhizobacteria (PGPR) inoculated seedlings were taller than those inoculated with *Rhizopogon parksii* from Oregon (RPAR-OR) (Figure 4).

Figure 4. Miriam Creek: Douglas-fir seedling height after 1 year



We collected growth data on all seedlings at Miriam Creek in September 2001. We have yet to do the data analysis, but based on averages only, it appears as though the major growth difference is between the cutblock (best growth) and burn pile trees and the trees on the landings (Table 3). Growth on the landings is clearly poorer than on the cutblock or burn pile. Based only on averages, there are no clear differences between the different biological inoculation treatments.

Table 3. Total shoot height in September 2001, 2 growing seasons after outplanting of Fdi at Miriam Creek on cutblocks and landings (averages only).

Biological inoculation treatment	Rehabilitation treatment			
	Burned Pile	**Cut Block**	**Deep tilled landing**	**Scratched landing**
Control	356	364	256	248
Laccaria laccata		372	256	255
PGPR	347	364	257	244
Rhizopogon BC	323		245	231
Rhizopogon OR		382	257	239
Overall	**342**	**370**	**254**	**243**

In addition, we have destructively harvested a subset of seedlings to assess root growth and ectomycorrhiza development. The mycorrhiza assessment has been carried out by Francois Teste, M.Sc. candidate at Simon Fraser University under the supervision of Margaret Schmidt. The participation of Francois in this project brought the advantage of additional human and

financial resources (Dr. Schmidt and SFU are providing support to Francois) and the opportunity for training a graduate student.

Establishment of trial at Fort St James

In collaboration with Apollo Forest Industries, UNBC, and the Fort St James District, we installed trials with Fdi, Sx and Pli using the biological treatments described in Table 4. In June 2001, the seedlings were planted into unrehabilitated winter roads, remains of burn piles, and cutblocks. Because, as described above, there was no evidence of colonization in response to inoculation of Fdi, we opted to plant only uninoculated control seedlings. Although percent colonization of Sx and Pli did not increase in response to inoculation, we outplanted these seedling treatments because the inoculated fungi may have supplanted the indigenous nursery fungi while the percent colonization remained the same.

Table 4. Biological treatments applied to seedlings planted in the Fort St. James District.

Plant species	Ectomycorrhizal inoculants	PGPR
Fdi	Control	
Sx	Control *Hebeloma longicaudatum* *Rhizopogon subcaerulescens*	
Pli	Control *Hebeloma longicaudatum* *Rhizopogon rubescens*	

New studies

New study to improve rooting and mycorrhiza development of Fdi

Guoping Xiao assessed root and mycorrhiza development of 10 randomly selected seedlings from each of the treatments: three different growing substrates (fine, medium and coarse textured) and 2 different fertilizer regimes (soluble fertilizer only, soluble and slow-release fertilizer combined). The seedlings were assessed at time of lifting when the seedlings were prepared for cold storage. He found no differences between any of the treatments; none of them were mycorrhizal.

There are many possible interpretations of this: Fdi just does not form fine feeder roots and ectomycorrhizae when it is very young; Fdi does not form fine feeder roots or ectomycorrhizae in container nurseries; we did not try enough manipulation of treatments to bring about the desired effect. In a previous study at the Research Branch Laboratory, we took cold stored container stock of Fdi from the same nursery and potted it on in a soil:sand mix. All of the Fdi formed abundant ectomycorrhizae within months, suggesting that it is not so much age as cultural conditions affecting root and mycorrhiza development.

New study to assess native mycorrhiza potential of Miriam Creek site

Francois Teste, M.Sc. student at SFU, carried out a bioassay of native ectomycorrhiza potential in the landings at the Miriam Creek site. The greenhouse bioassay was a fully randomized design with 120 container-grown Douglas-fir seedlings. The originally nonmycorrhizal seedlings from Riverside Nursery were grown in soil collected from the

landings. Rhizopogon and 7 other morphologically distinct ectomycorrhizal fungi heavily colonized seedlings from the nursery. These results suggest that Douglas-fir seedlings can readily form ectomycorrhizae under harsh conditions.

EXTENSION

Shannon Berch and Chuck Bulmer have presented summaries of this work to District and company foresters as part of site rehabilitation field tours in Prince George and Robson Valley Forest Districts in June and October, 1999, respectively.

Shannon Berch was invited to make a presentation on mycorrhizae in commercial nurseries and on this project to the 19th Meeting of the Forest Nursery Association of British Columbia in Vancouver, B.C., in September of 1999.

Chuck Bulmer presented our results as part of a paper and related poster to the "Science to Management" workshop held in December 1999.

Chuck Bulmer included a discussion of this project in his recently published Soil conservation 'Info-flip'.

We also prepared and submitted an article for Seed and Seedling Extension News (in press). Because of the truncation of funding, we have yet to prepare the refereed journal articles, but this will begin to happen in 2003/4. Publication of field responses will occur once the field data is collected.

In late spring/summer Francois Teste will commence writing his Master's thesis at SFU based on Fdi from Miriam Creek including mycorrhizae, growth, and nutrient content.

SUMMARY AND CONCLUSIONS

Lodgepole pine container stock in BC nurseries is highly mycorrhizal without inoculation and interior spruce is just slightly less so. This does not mean that the mycorrhizal fungi occupying the roots will give the seedling an advantage when outplanted because indigenous fungi often replace nursery fungi. But, given the occupation of roots by ectomycorrhizal fungi, such as *Thelephora terrestris*, that are adapted to nursery conditions, it may be difficult for inoculated fungi to compete. The situation is different for Douglas-fir because this species is so poorly colonized by nursery fungi that there is the potential for an inoculated fungus to succeed.

However, from the inoculation study, we conclude that mycorrhizal inoculation in the container nursery with the inoculants tested does not reliably improve percent ectomycorrhizal colonization of Douglas-fir, lodgepole pine or interior spruce. Clearly there is additional research to be done if Fdi development in the nursery and survival and growth after outplanting are to be improved. We conclude that there is real potential for inoculation of Douglas-fir if procedures, such as altering the form or rate of application of fertilizers, can be standardized to the point that an improvement in colonization can be assured.

We don't yet know whether inoculation improves field performance, despite the lack of difference in colonization, because our field trials are still being monitored.

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STATEMENT OF EXPENDITURES

See page submitted separately by UBC.